Pattern Recognition Receptors

Seeing and responding to danger

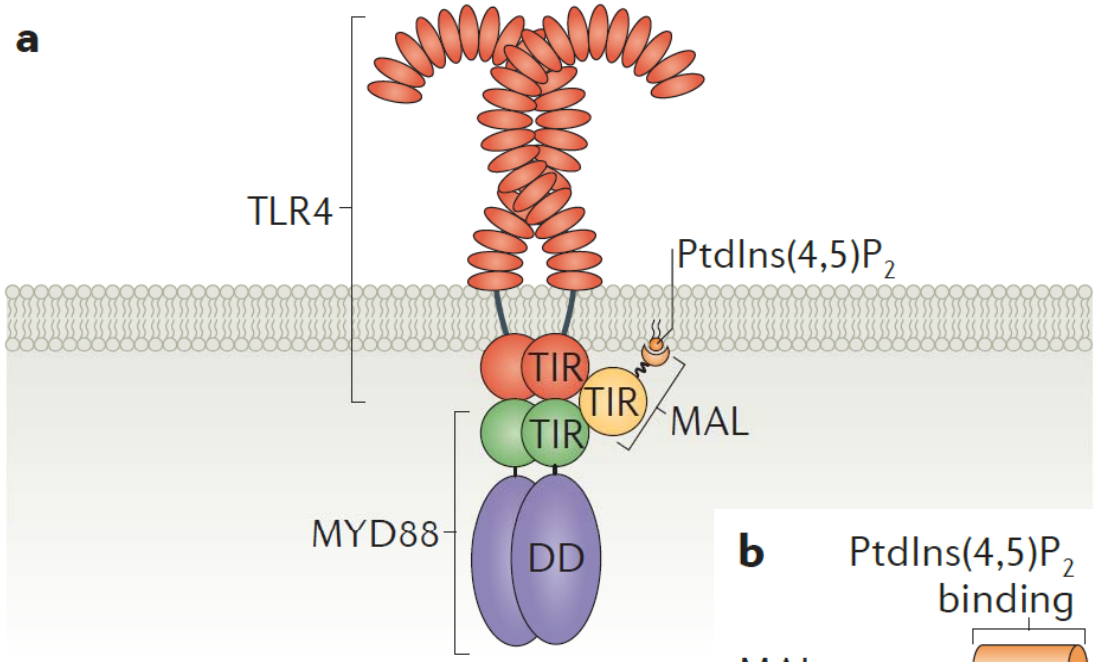
Tom Monie

Lecture 2

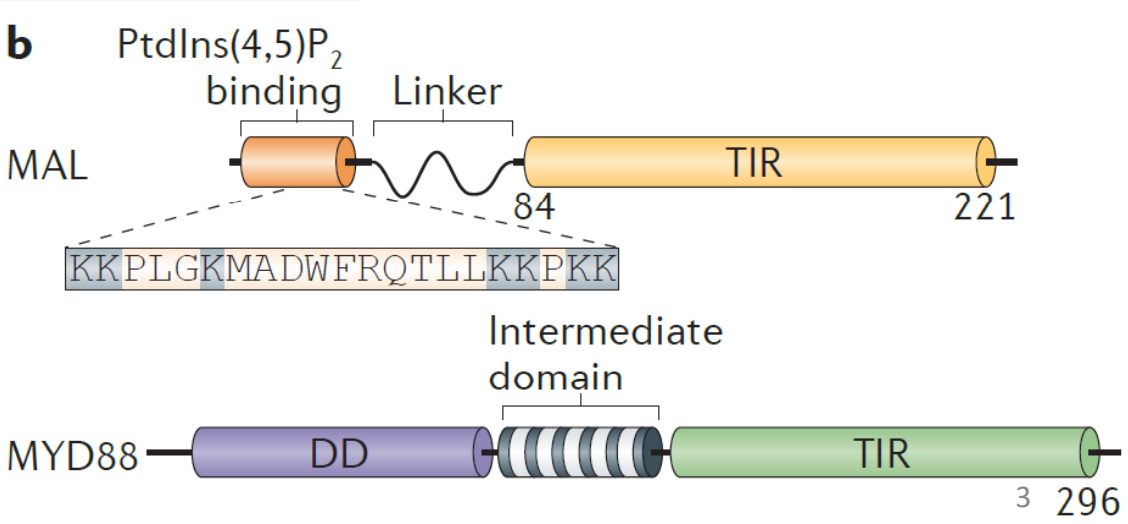
Lecture overview

- PRR signalling pathways
  - Activation and signal transduction
- Ligand recognition by TLRs and NLRs
- Macromolecular signalling complexes
  - Myddosome and inflammasome
- Detection of LPS
  - cell surface versus cytoplasm
- PRRs and disease

Multiprotein macromolecular PRR signalling complexes

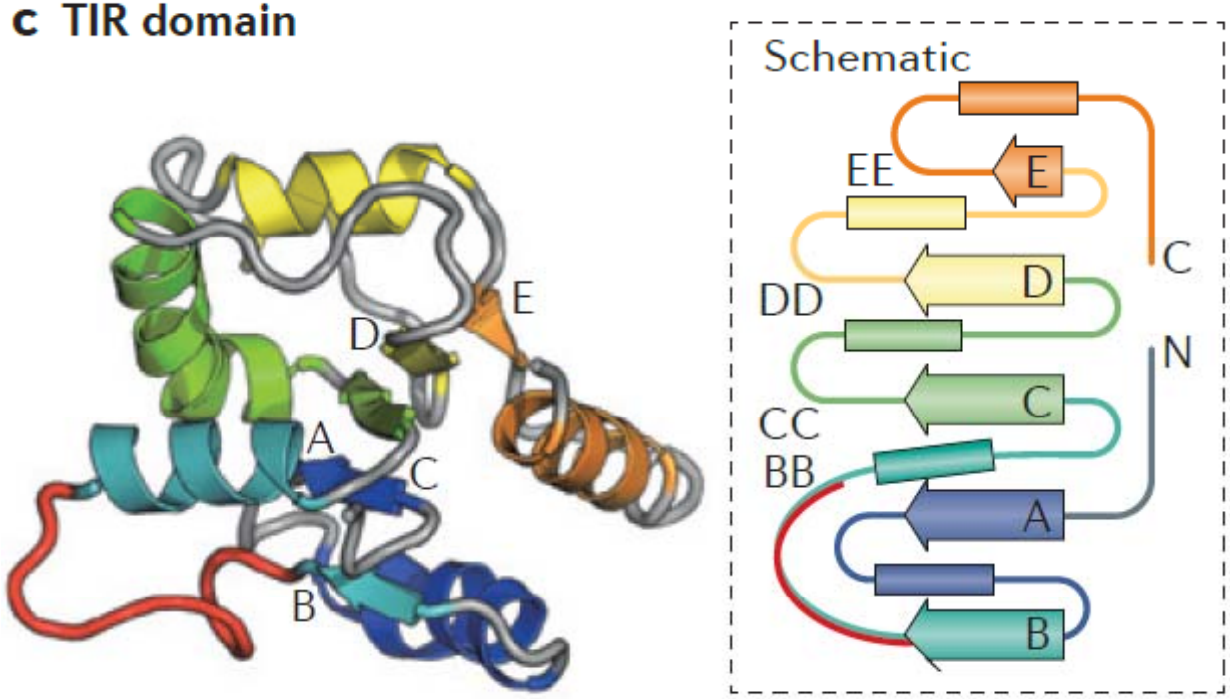


TLR signalling complexes are first built around TIR domain interactions



Gay et al Nat Rev Immunol 2014 14:546-558

Multiprotein macromolecular PRR signalling complexes

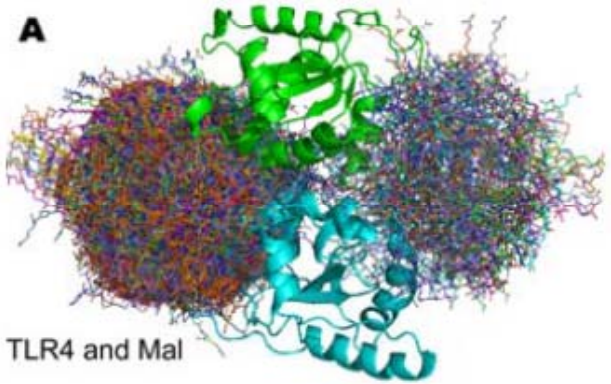


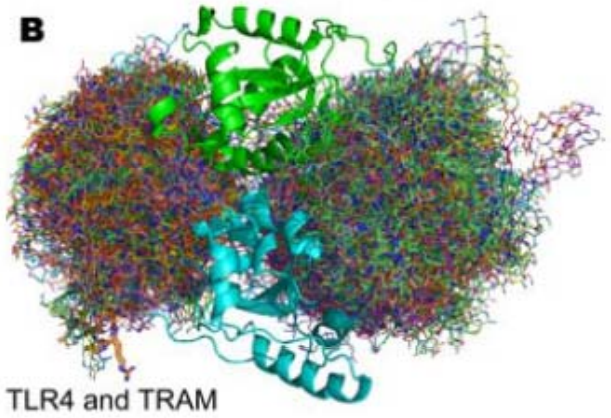
The BB loop is a key functional part of the TIR

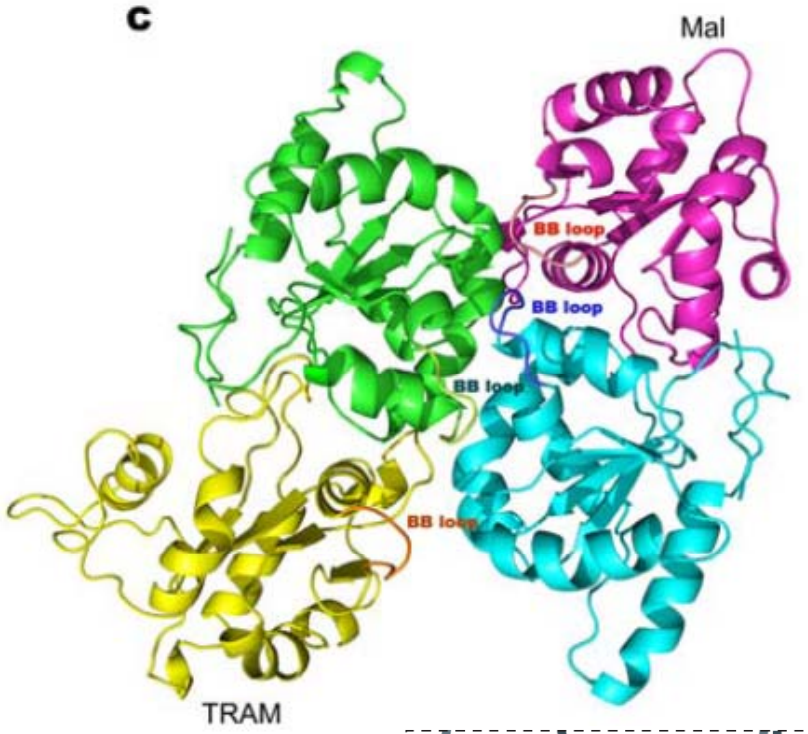
TLR1hs	LEKEG.....M	QICLHERNFV	PGKSIV
TLR1mm	NLEKDD.....I	QICLHERNFV	PGKSIV
TLR2mm	QQLENSDPP...F	KLCLHKRDFV	PGKWII
TLR2hs	QELENFNPP...F	KLCLHKRDFI	PGKWII
TLR3hs	SMEKED.....Q	SLKFCLRDFE	AGVFEE
TLR3mm	SPMEEQDQS...L	KFCLEERDFE	AGCLGLE
TLR4hs	LEEGVPP...F	QLCLHYRDFI	PGVAIA
TLR4mm	NLEEGVP....F	HLCLHYRDFI	PGVAI
Tolldm	QLEHGPQ....F	QLCVHERDWL	VGGHI

Gay et al Nat Rev Immunol 2014 14:546-558
PNAS 2007 104 (18) 7506-7511

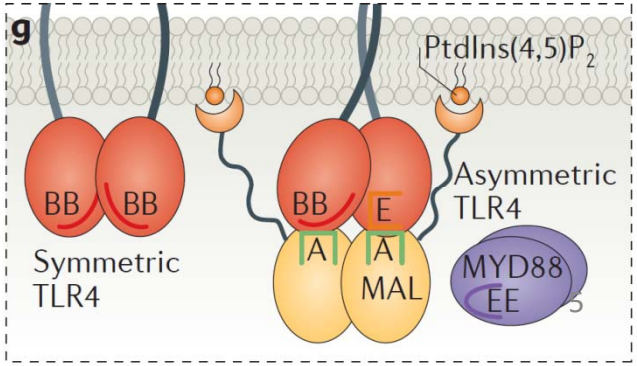
Multiprotein macromolecular PRR signalling complexes





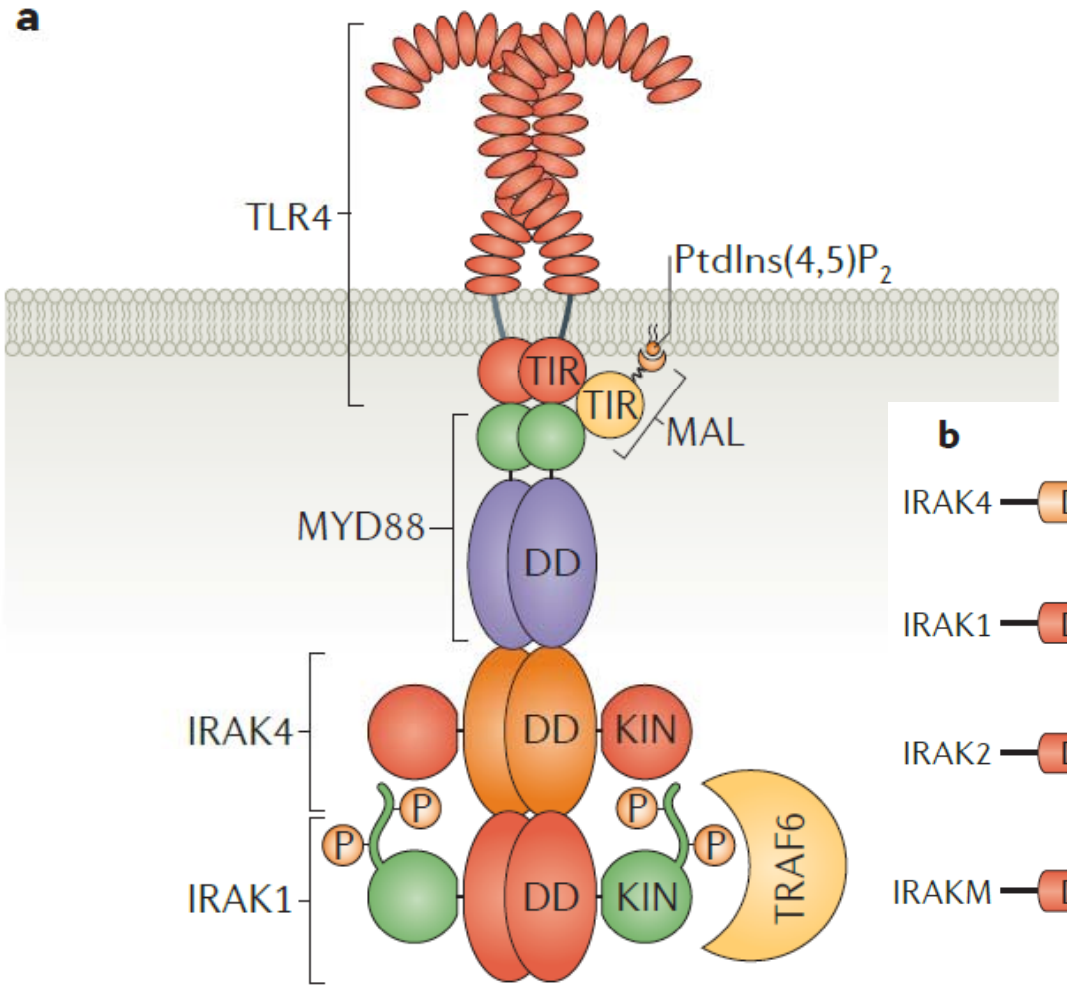


Interactions may be symmetric of asymetric

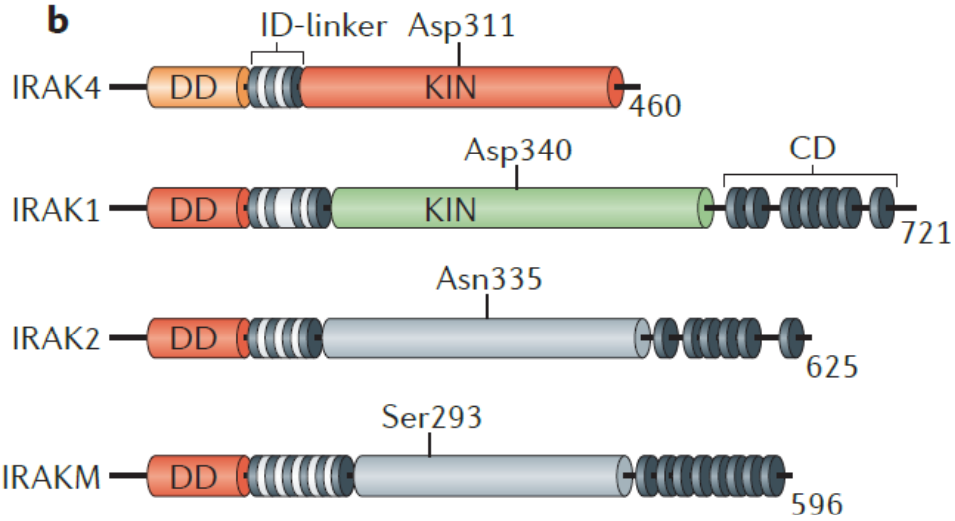


Nunez Miguel et al PLoS One 2007 8:e788 & Gay et al Nat Rev Immunol 2014 14:546-558

Multiprotein macromolecular PRR signalling complexes – the Myddosome

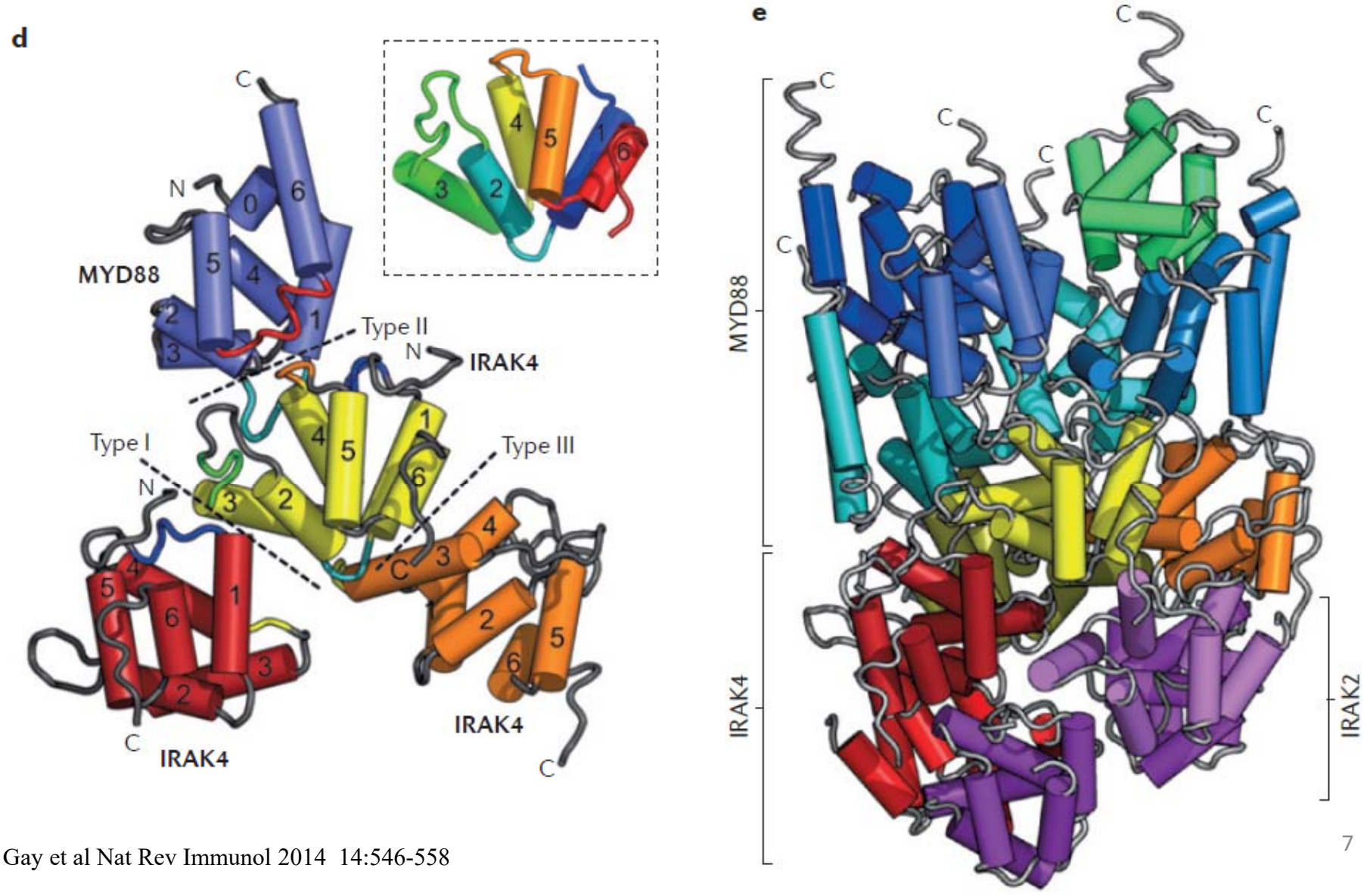


After the TIR:TIR interactions come the Death domain: Death domain complexes



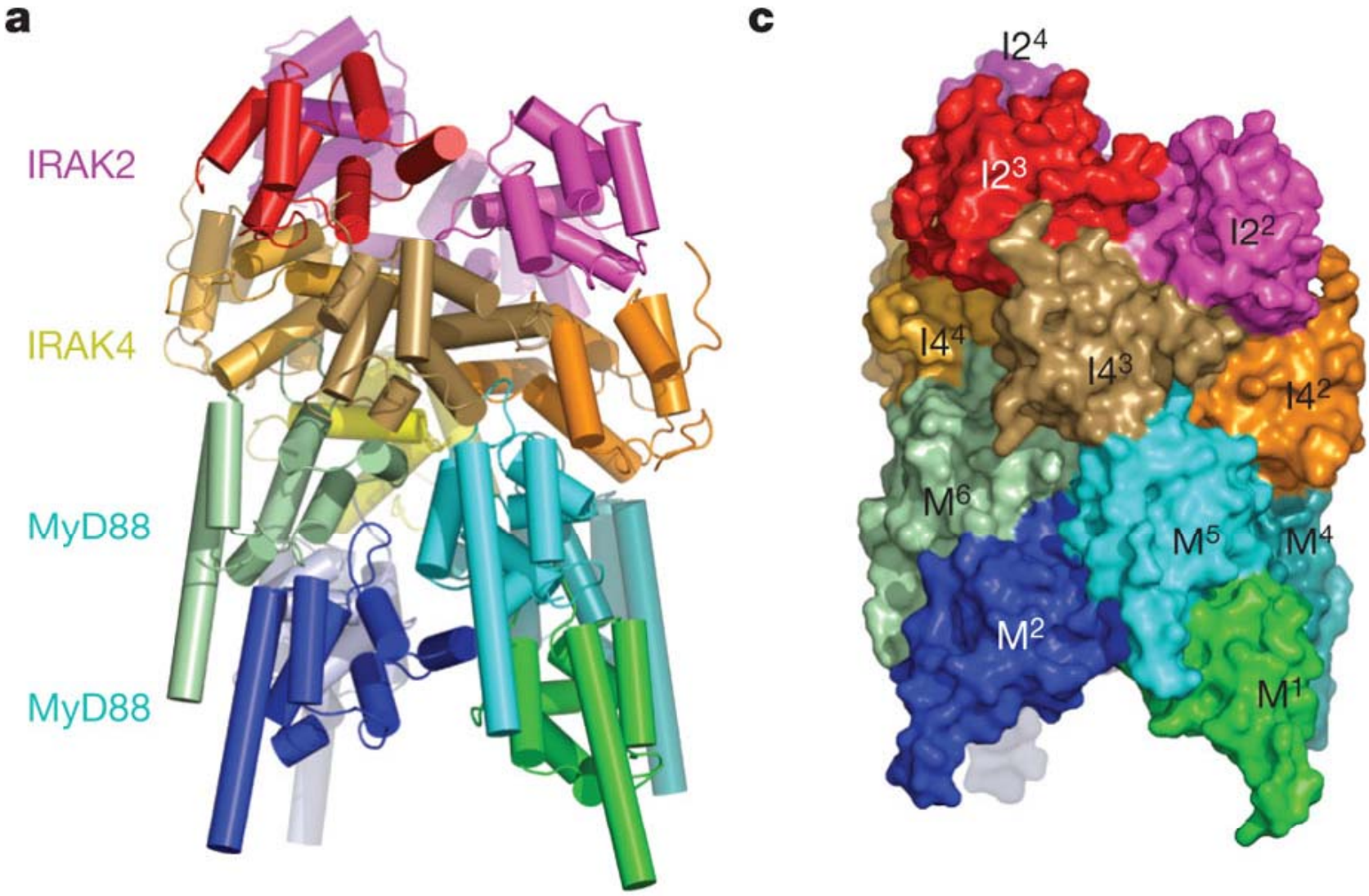
Gay et al Nat Rev Immunol 2014 14:546-558

The Myddosome



Gay et al Nat Rev Immunol 2014 14:546-558

The Myddosome



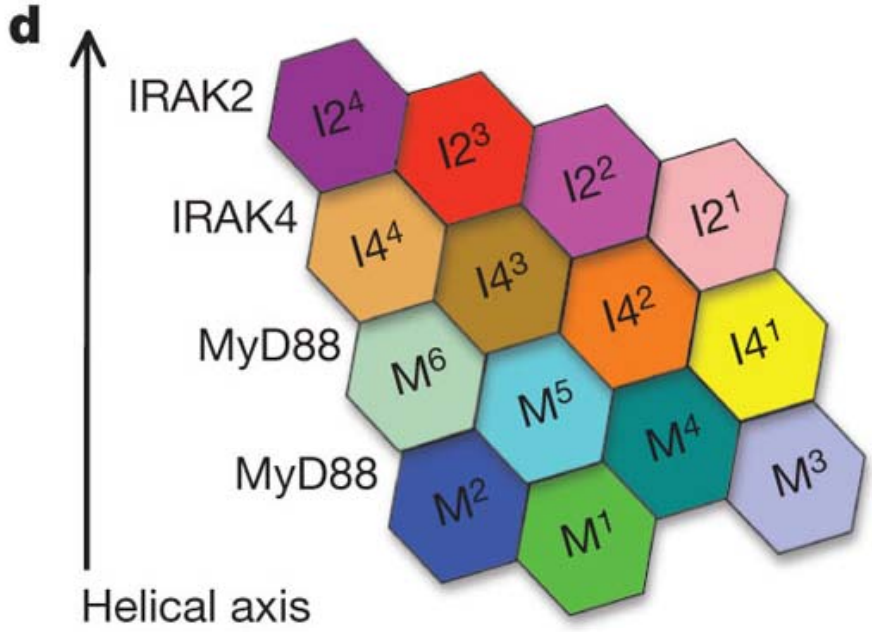
Lin et al 2010 Nature **465**, 885–890 doi:10.1038/nature09121

The Myddosome

The Myddosome has a helical conformation – common in death domain complexes, especially overexpressed ones.

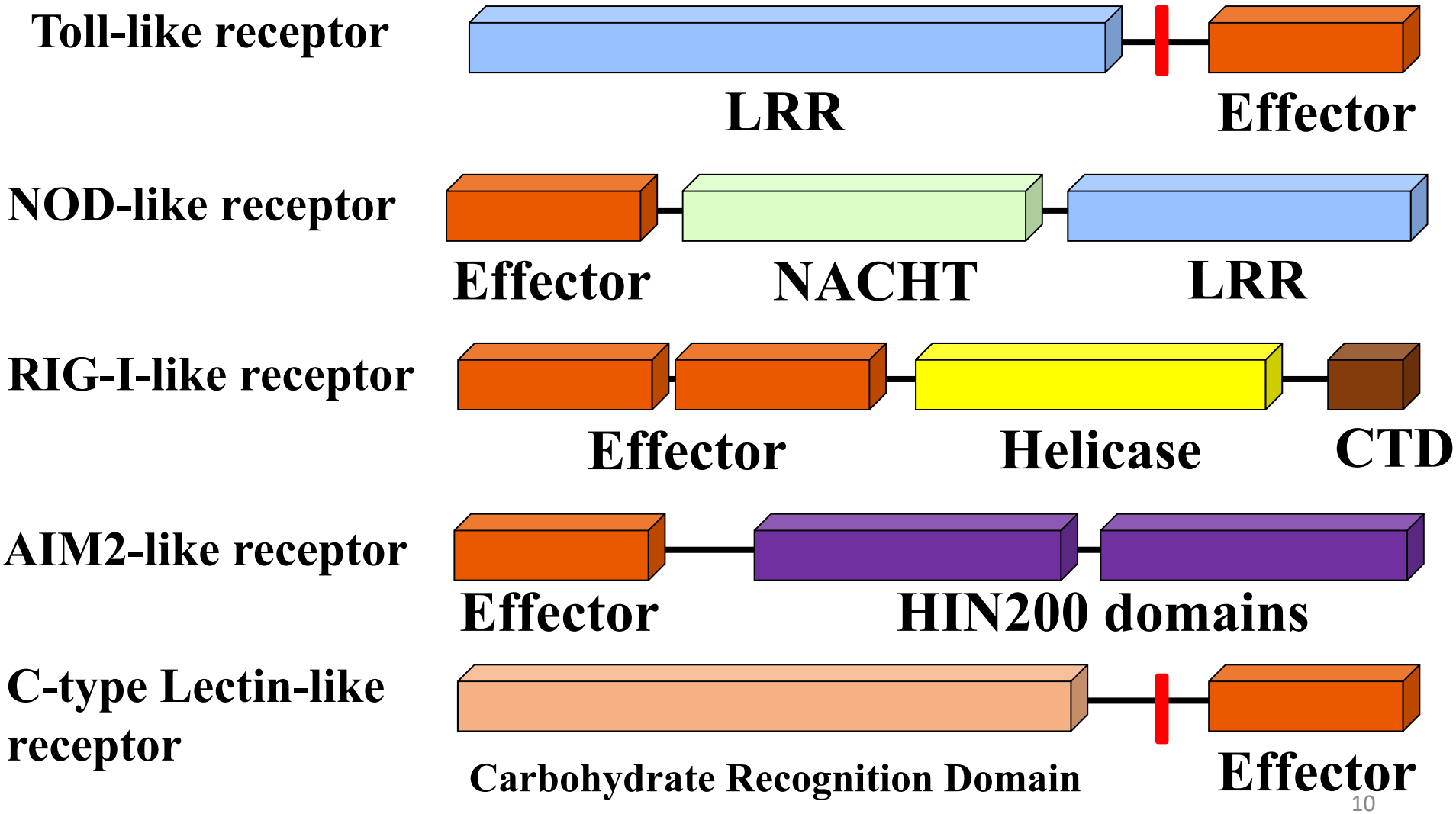
There is ongoing debate about the complex stoichiometry (MyD88:IRAK4 - 7:4 or 8:4)

The actual complex in the cell may be much much bigger.

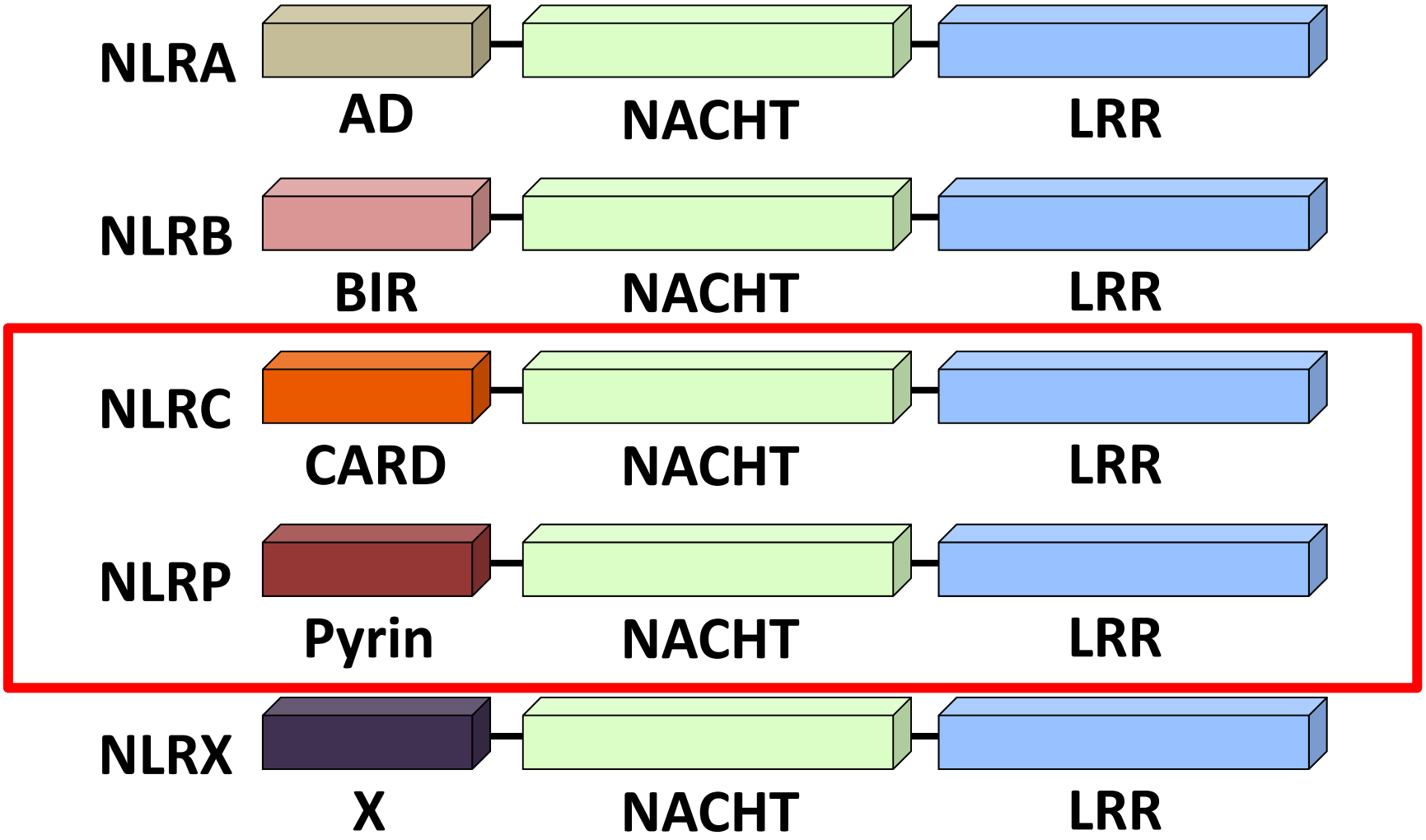


Lin et al 2010 Nature **465**, 885–890 doi:10.1038/nature09121

PRR Domain Structure



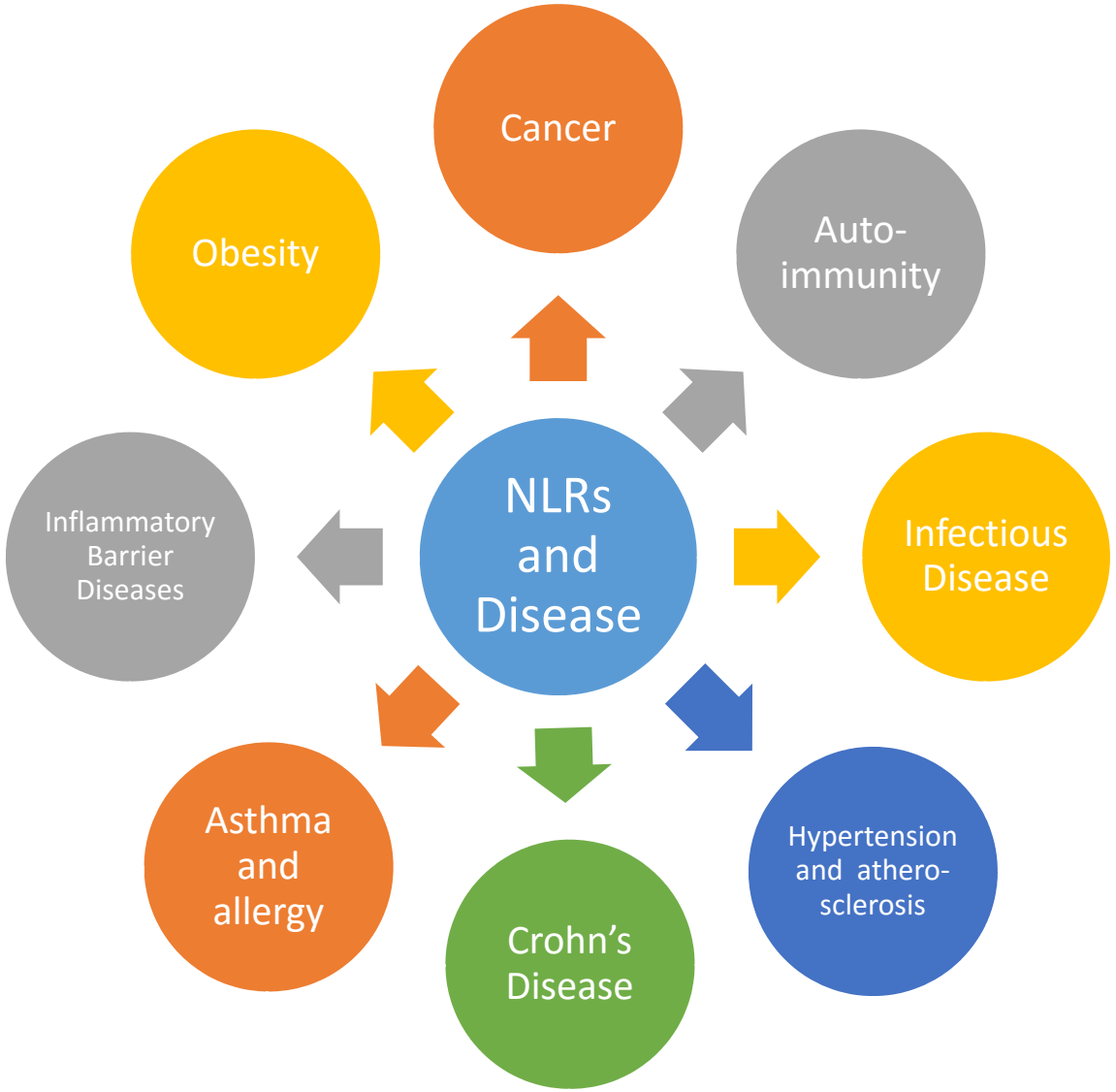
The NLR family of PRRs



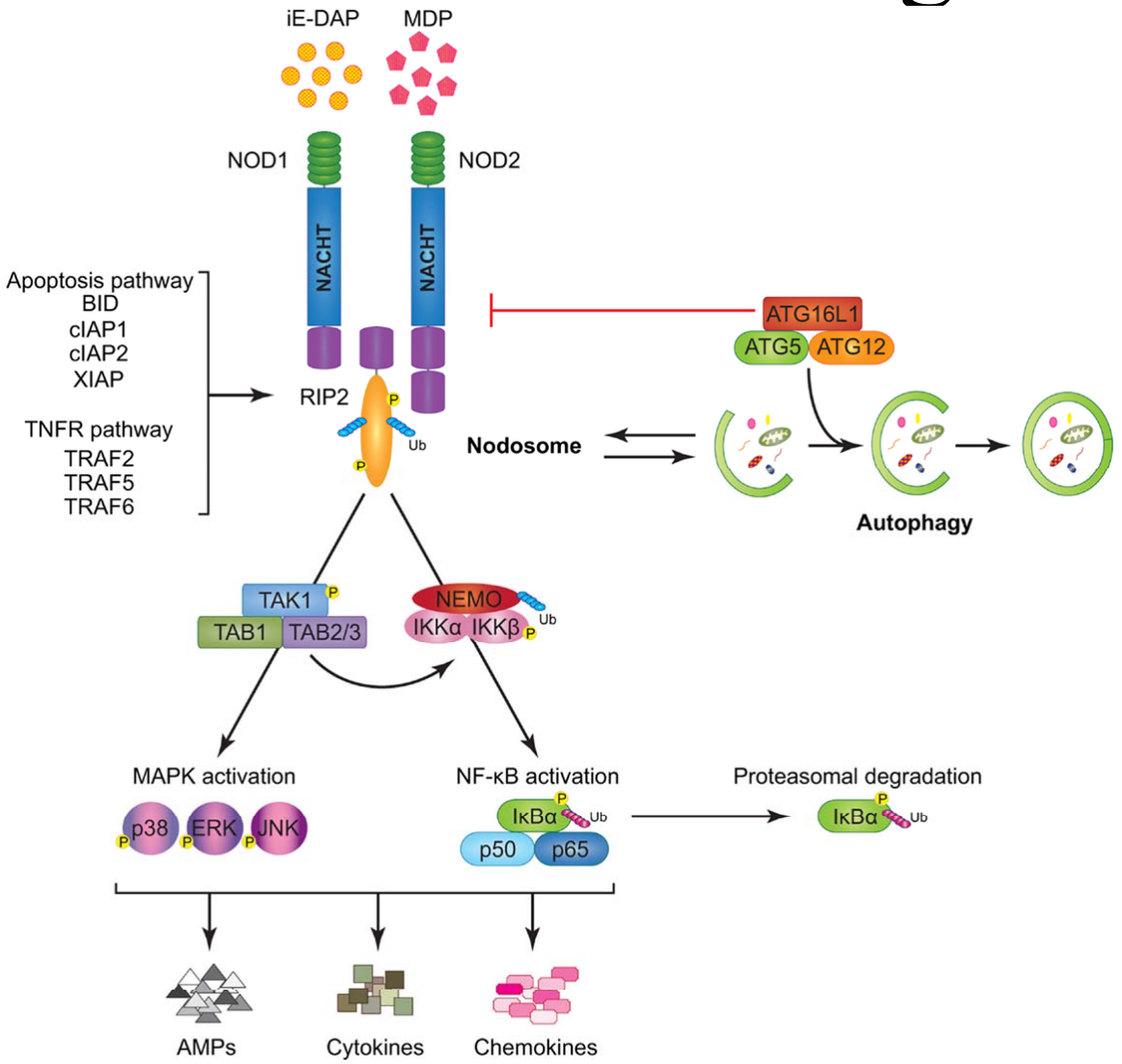
What do the NLR family do?

- Upregulate a pro-inflammatory immune response via NFkB and stress kinase pathways (NOD1, NOD2)
- Form a large multiprotein complex (inflammasome), resulting in caspase-1 processing and IL-1b and IL-18 secretion (NLRC4/NAIP, NLRP1, NLRP3
- Immunoregulation – transcriptional control of MHC-I (NLRC5) and MHC-II (CIITA)
- Roles in development
- Rich diversity in cross-species NLR repertoire

Why the interest in NLRs?

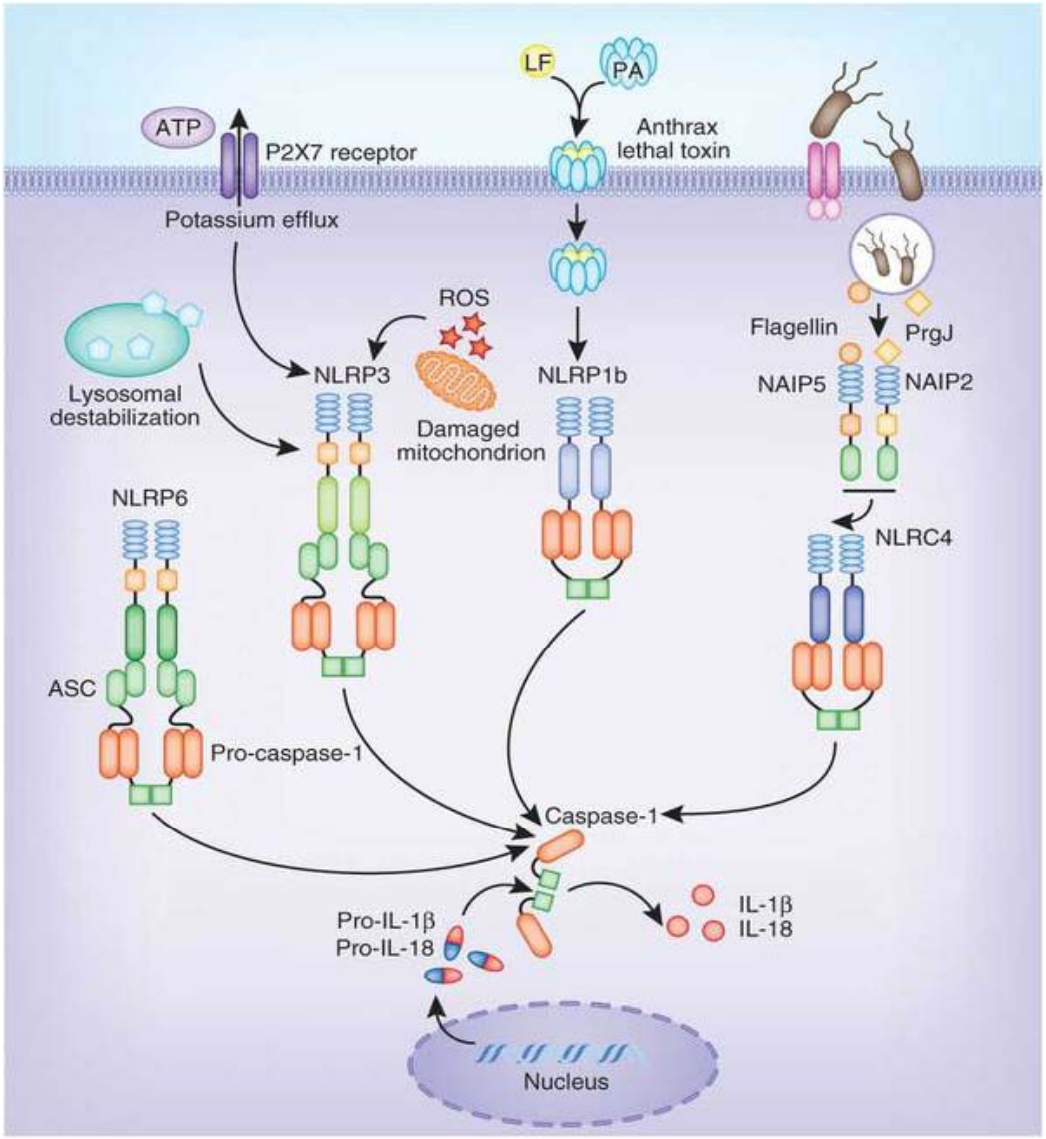


NOD1 and NOD2 signalling



Saxena & Yeretssian (2014) Front. Immunol. 10.3389/fimmu.2014.00327

Inflammasome signalling

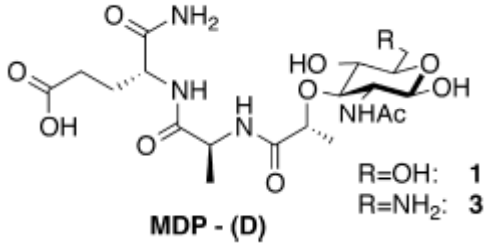


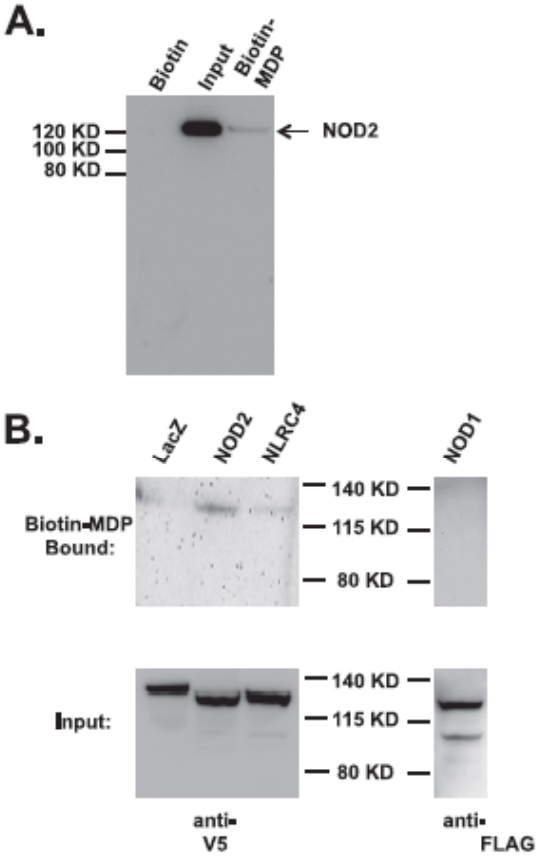
Rathinam *et al.,* (2012) Nat. Immunol 13 333-342

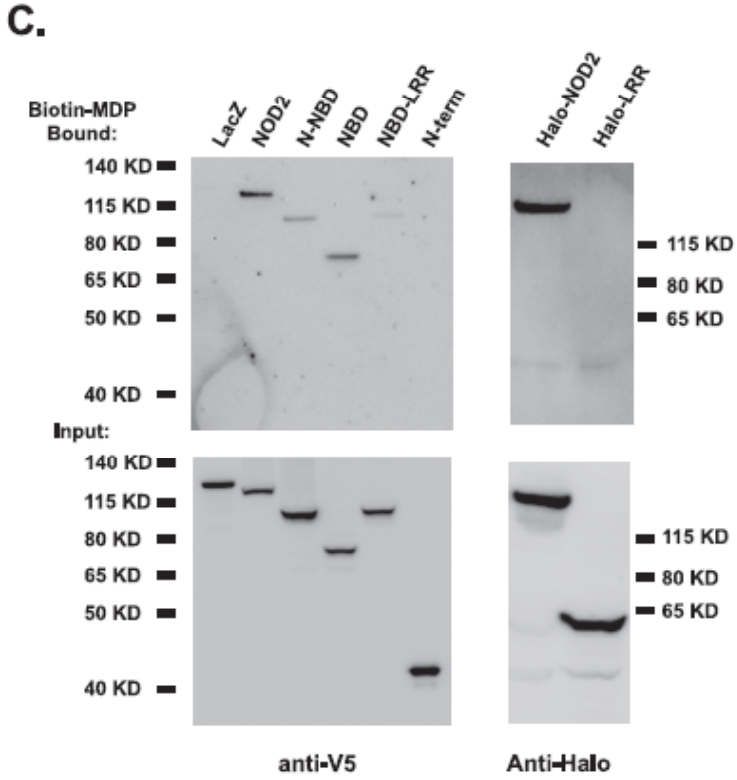
NLR activation

- Direct ligand binding
  - NOD1 (ie-DAP)
  - NOD2 (Muramyl dipeptide)
  - NLRC4/NAIP – FliC (flagellin), Type 3 secretion systems rod proteins e.g. PrgJ
  - NLRX1 – ssRNA dsRNA
- Indirect activation or an unidentified direct ligand
  - NLRP3 – ATP, Uric acid, Nigericin, cholesterol, alum, silica, pore-forming toxins, potassium efflux, mitochondrial DNA ....

NLR activation – NOD2







Muramyl dipeptide binds directly to NOD2, but which domain is important?

Mo *et al.*, (2012) JBC 287(27): 23057-67
Grimes *et al.*, (2012) JACS 134(33): 13535-7

NLR activation – NLRX1

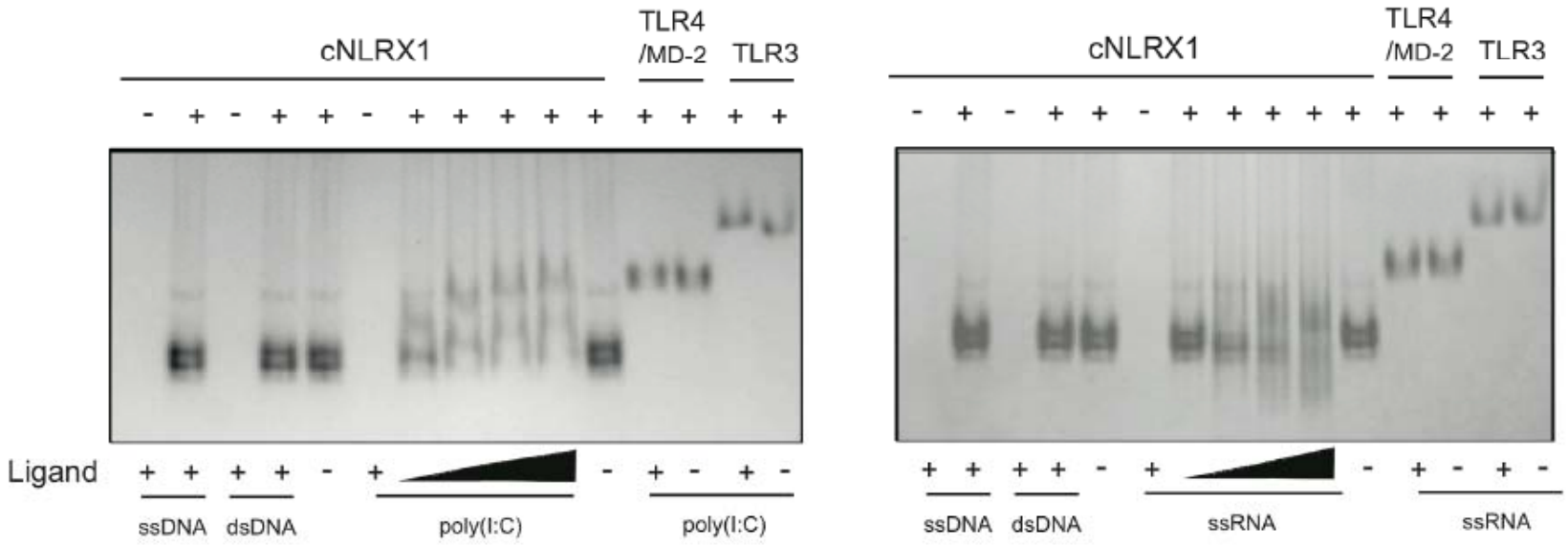


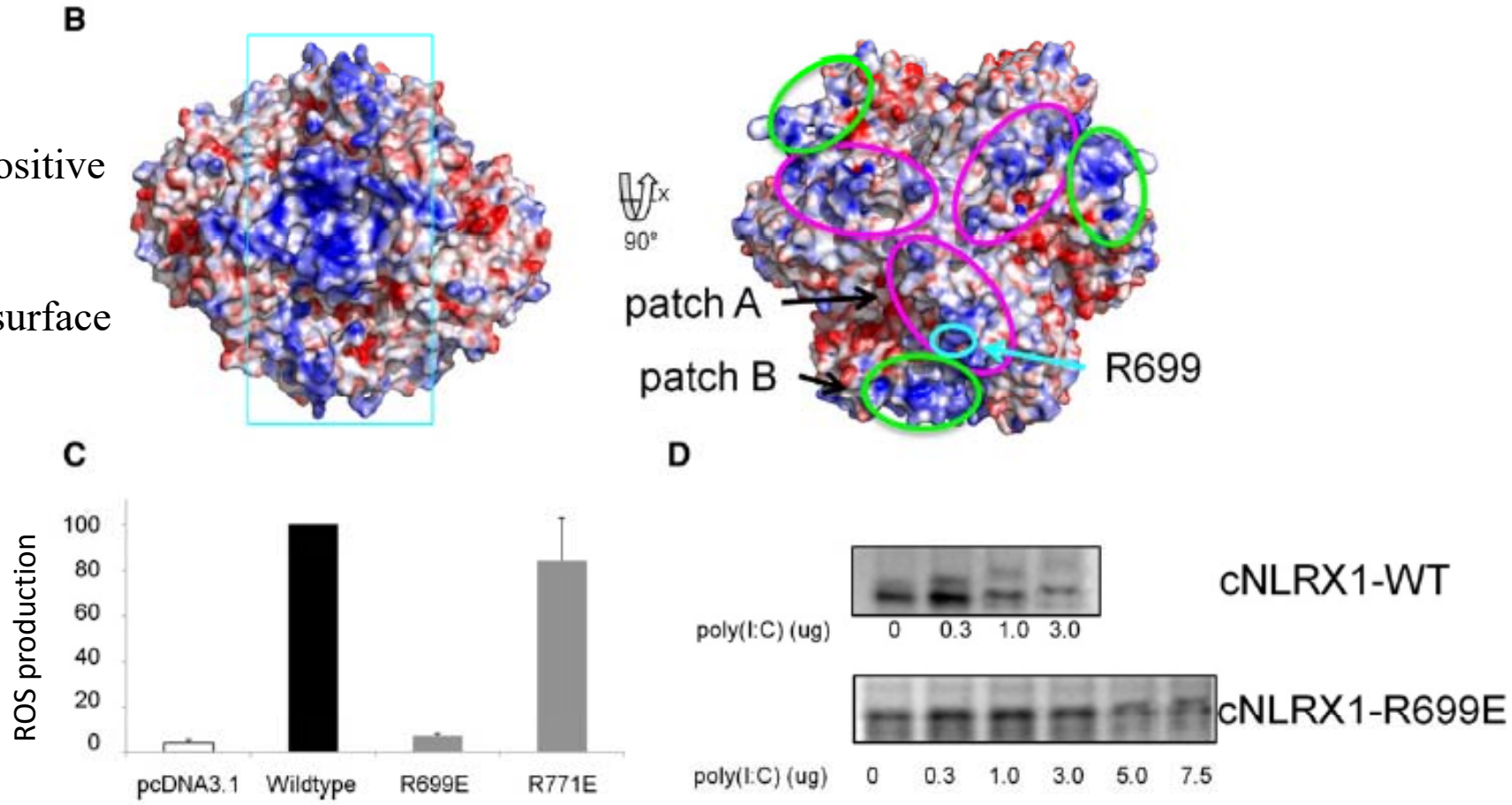
Figure S4. cNLRX1-RNA interaction.
cNLRX1 interaction with viral RNA mimic, poly(I:C) (left) and ssRNA (right) was analyzed by native PAGE. The extracellular LRR domain of TLR4 in complex with MD-2 was included as a negative control. The extracellular LRR domain of TLR3 that binds only to dsRNA was included in the analysis as positive and negative controls for poly(I:C) and ssRNA binding, respectively.

Binds both ssRNA and dsRNA (poly(I:C))

Hong *et al.,* (2012) Immunity 36(3): 337-347

NLR activation – NLRX1

Electropositive patch = potential binding surface

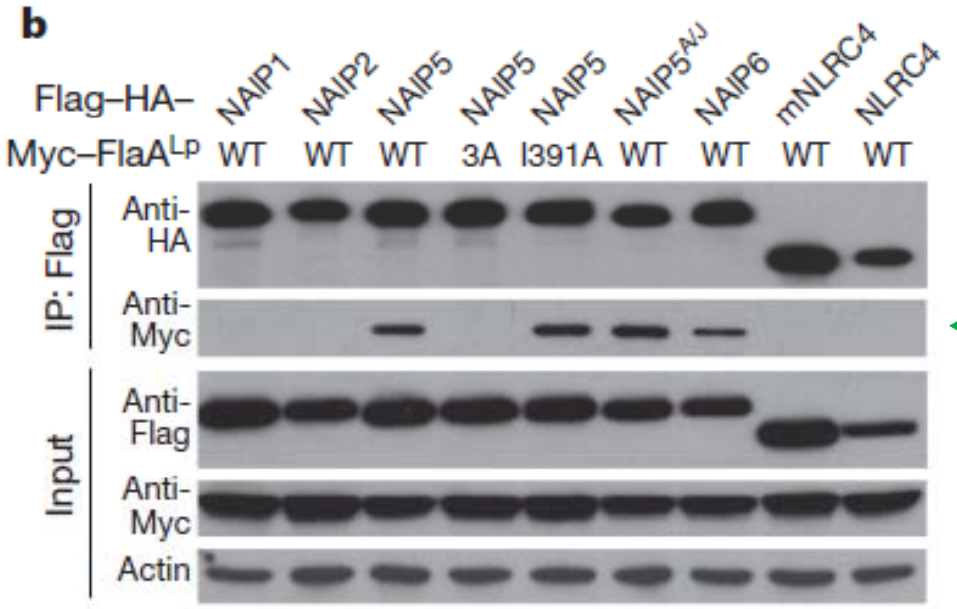


R699, but not R771, crucial to interaction with RNA ligand

Hong *et al.,* (2012) Immunity 36(3): 337-347

NLR activation – NLRC4/NAIP inflammasome

NLRC4 functions in conjunction with another NLR (NAIP) to form a functional inflammasome. Humans have a single NAIP, mice have a number of orthologs of which NAIP1, NAIP2, NAIP5 and NAIP6 are functional.



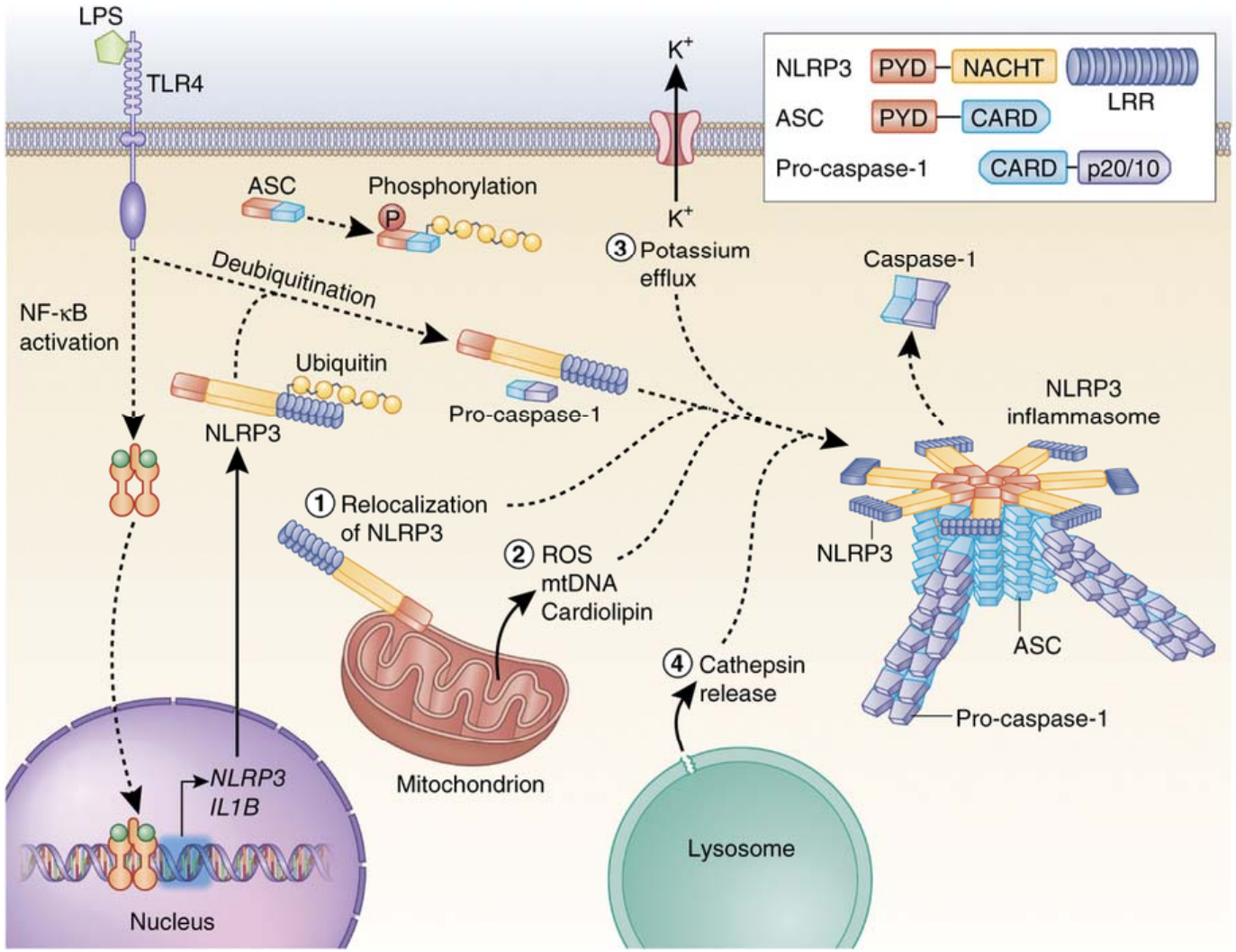
Zhou *et al.,* (2011) Nature 477: 596-600

The NAIP protein provides the ligand specificity

NAIP5 and NAIP6 recognise flagellin

NLRC4/NAIP also activated by Type 3 secretion system rod proteins (e.g. PrgJ)

NLR activation – NLRP3 inflammsome



Debbie Maizels/Nature Publishing Group

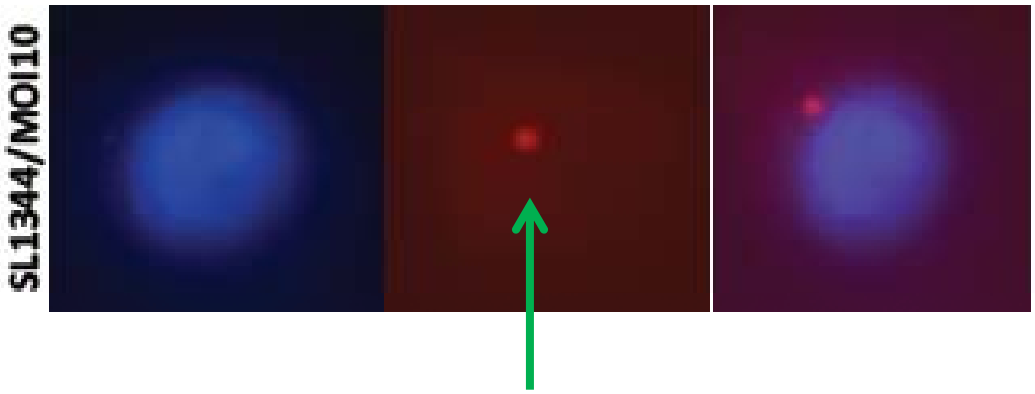
The NLRP3 inflammasome needs two signals:

Signal 1 – priming

Signal 2 – assembly and activation

Guo et al Nature Medicine 21, 677–687 (2015)

What does the inflammasome look like?



Inflammasome 'speck' formed upon infection of a macrophage with *Salmonella*

The inflammasome forms a large 'speck' in the cytoplasm. This may associate with specific organelles or cellular substructures.

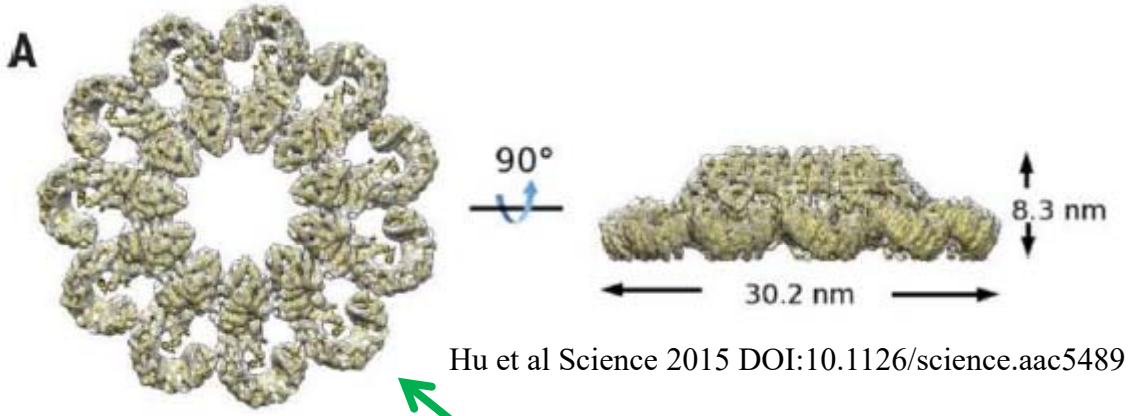
Inflammasome formation is driven by the protein:protein interactions that commonly involve members of the Death Domain superfamily.

The components of the inflammasome

- At the basic level the inflammasome consists of:
  - The adaptor protein ASC (CARD-Pyrin)
  - A relevant PRR (NLRP1, NLRP3, NLRC4/NAIP, AIM2)
  - Inflammatory caspases (Caspase-1)
  - Pro-interleukin-1β

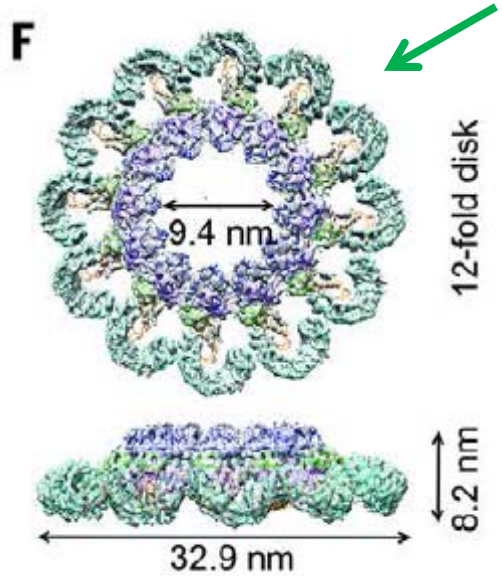
- **In reality it is more complex than this!**
  - **Multiple PRRs activated**
  - **More than one inflammatory caspase**
  - **Usually a single speck formed in each cell**
  - **Dynamic**

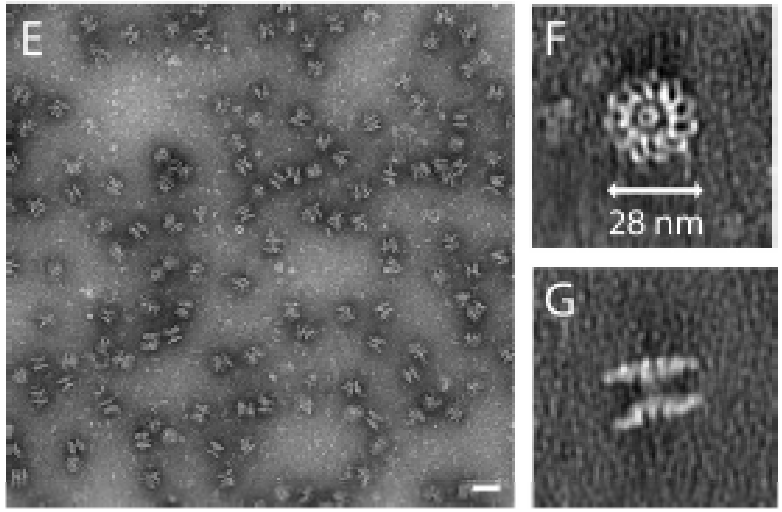
What does the inflammasome 'really' look like?



Hu et al Science 2015 DOI:10.1126/science.aac5489

PrgJ/NAIP2/NLRC4



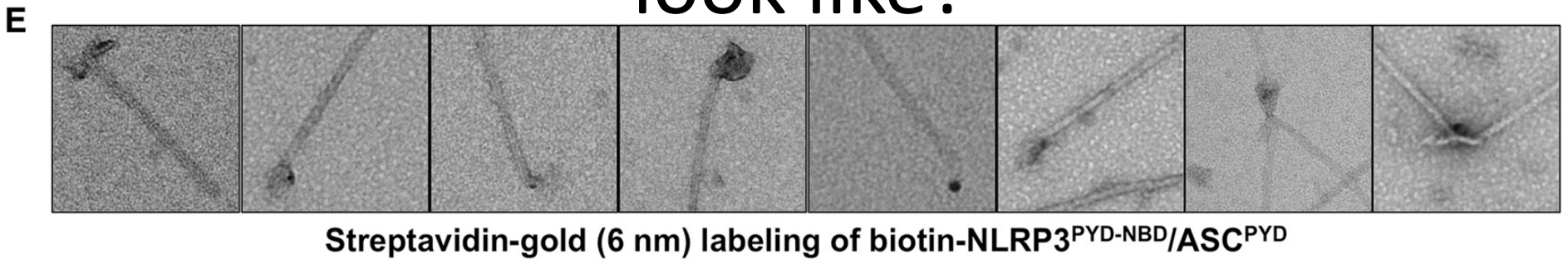


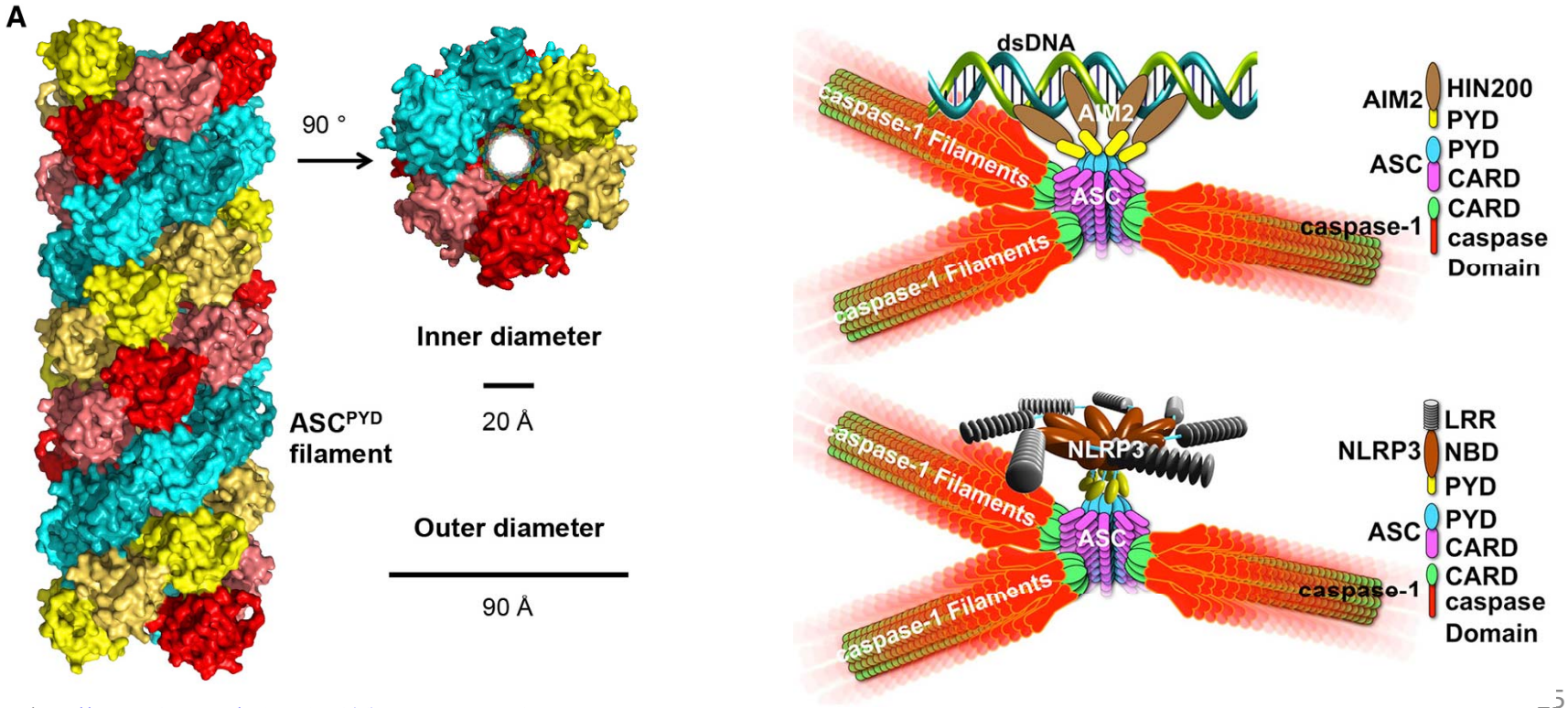
NAIP5/NLRC4

Zhang et al Science 2015 DOI:10.1126/science.aac5789

Halff et al Science 2012 287(46): 38460-38472

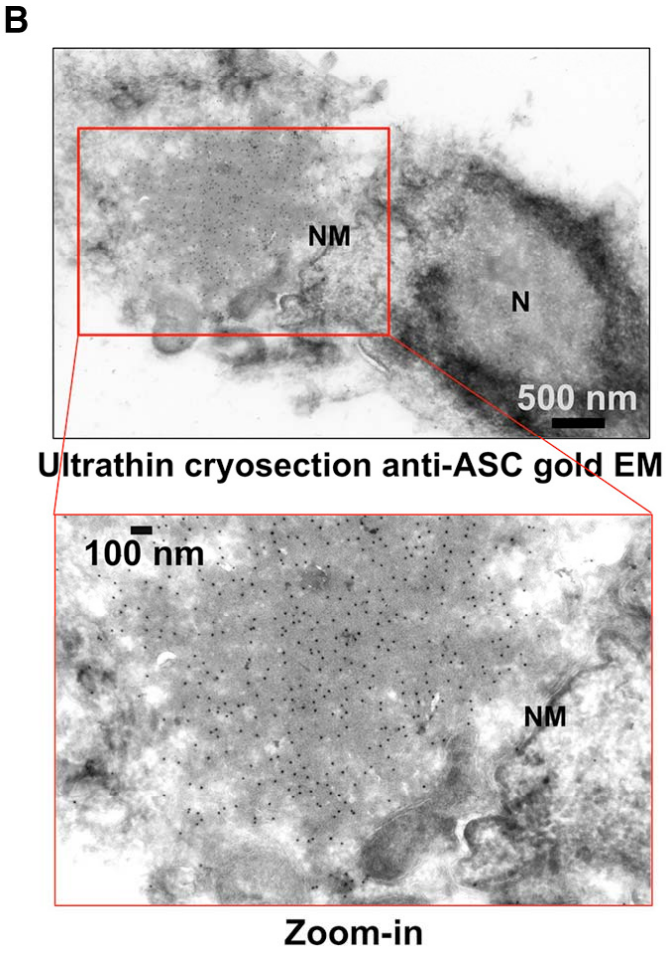
What does the inflammasome 'really' look like?





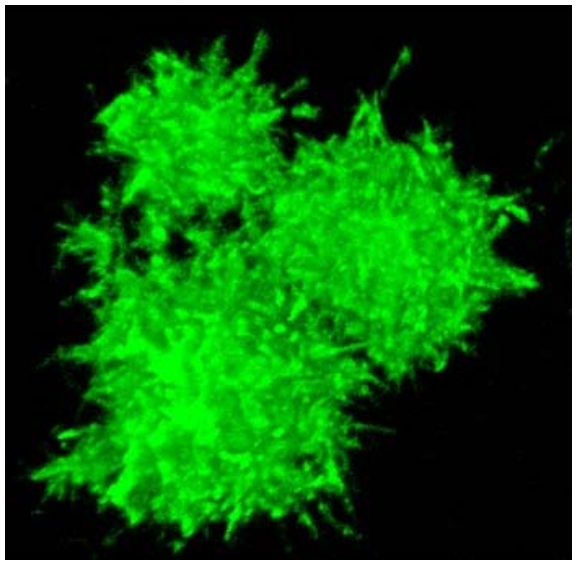
Lu et al Cell. 2014 March 13; 156(6): 1193–1206.

What does the inflammasome 'really' look like?



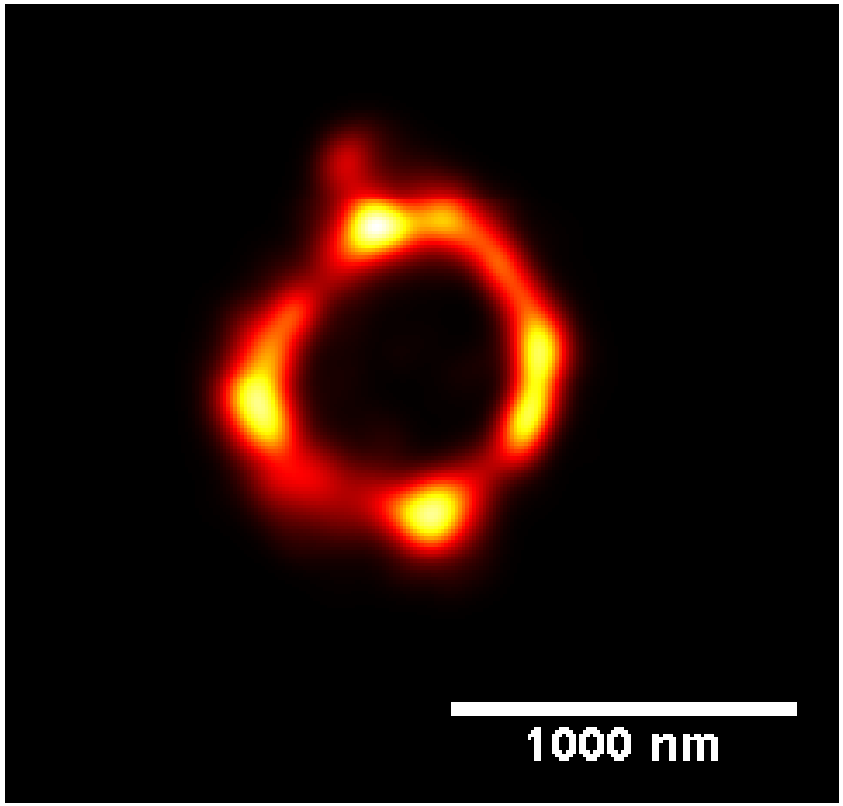
ASC specks forming filamentous aggregates

Lu et al Cell. 2014 March 13; 156(6): 1193–1206.



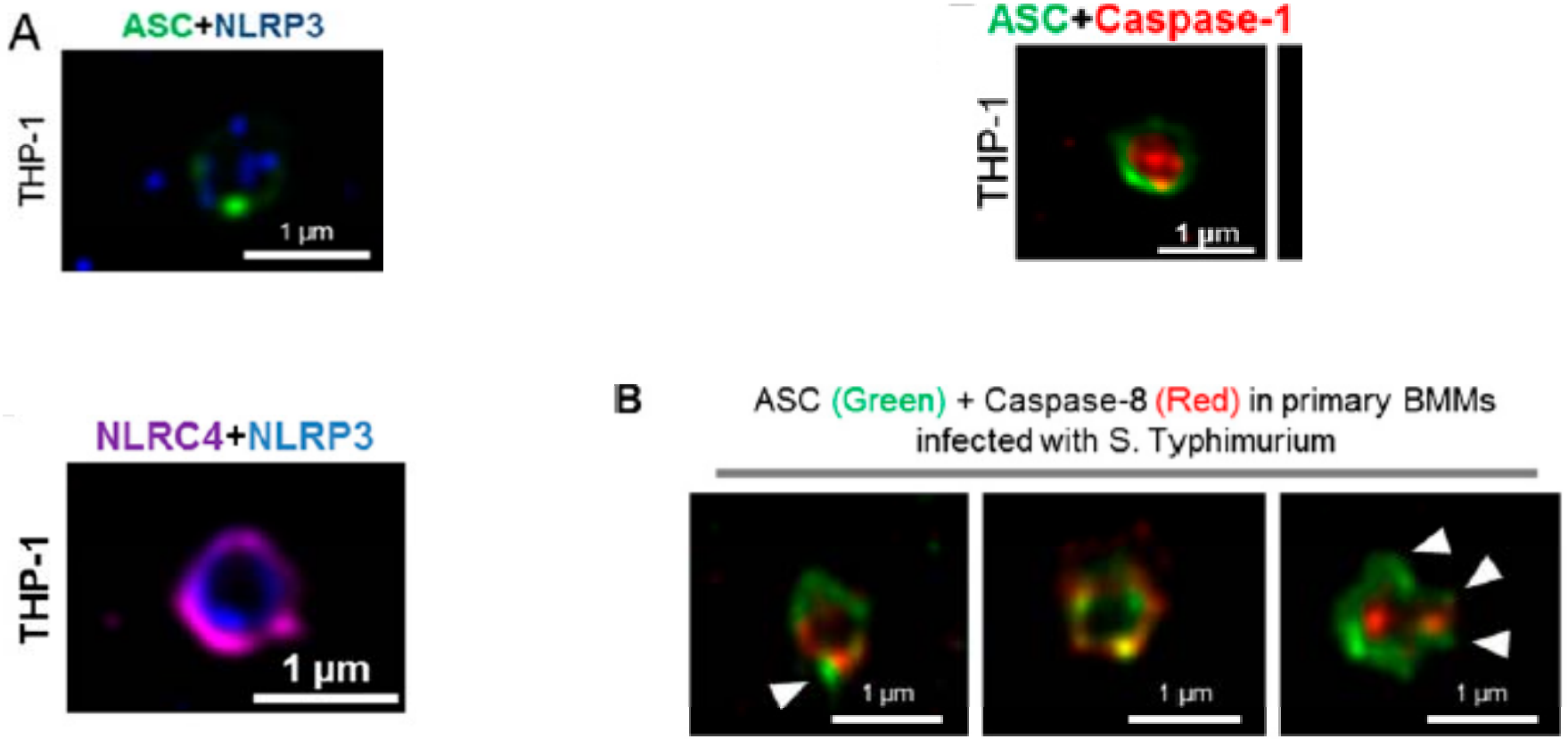
A hairy speck formed from overexpressed GFP-ASC (courtesy of E. Latz)

The real deal?



Endogenous inflammasome (anti-ASC) formed following *Salmonella* infection (Man et al 2014 PNAS 111(20) 7403–7408)

The real deal?

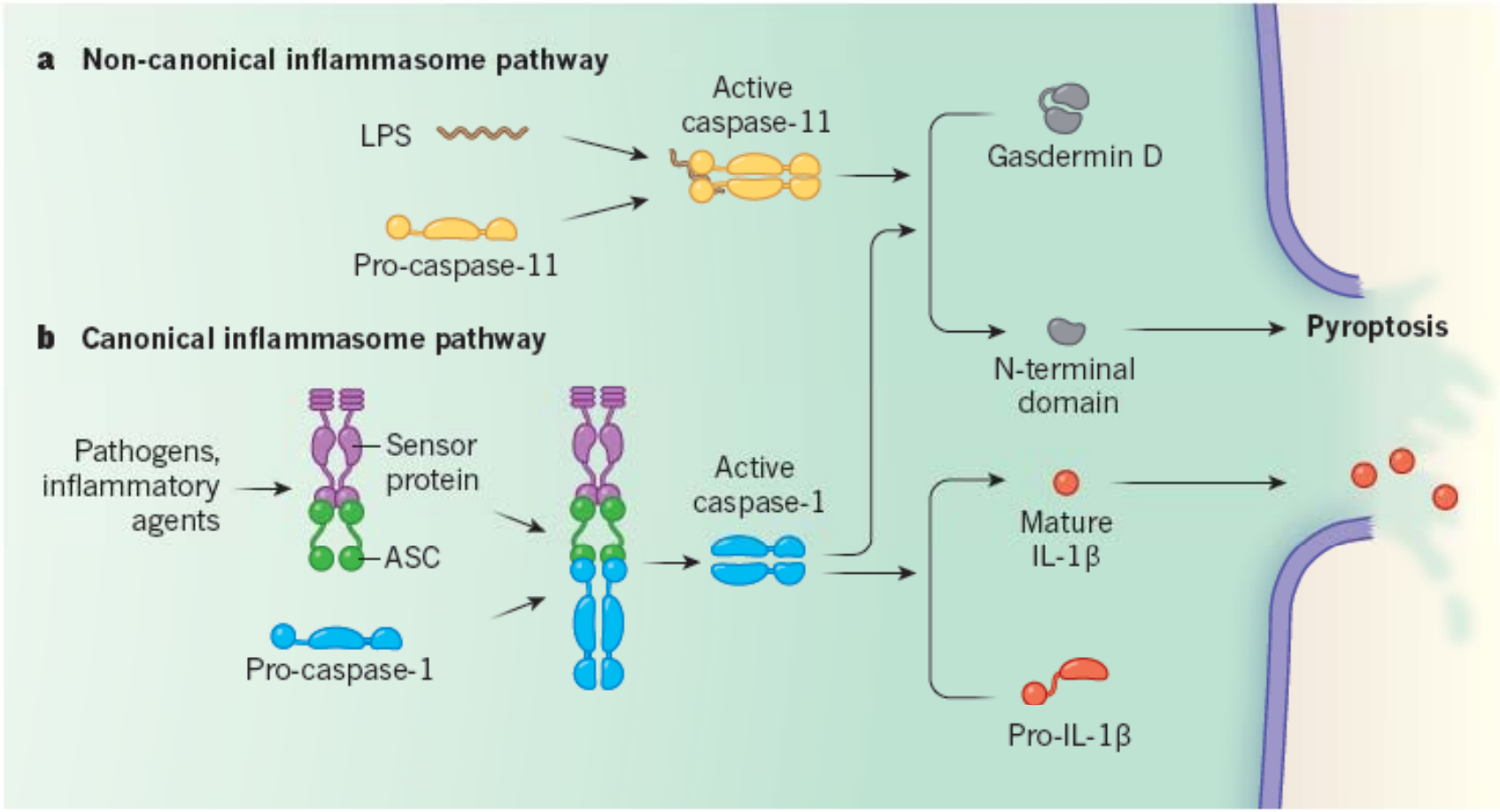


Multiple NLRs and multiple inflammatory caspases in the same speck

Cytoplasmic sensing of LPS – Caspase-11

- Extracellular LPS is detected by TLR4/MD2
- Intracellular LPS is recognised by the CARD of pro-caspase-11 (mice) or pro-caspase-4 or 5 (humans) (Shi et al Nature. 2014 Oct 9;514(7521):187-92)
- Caspase-11 stimulation results in cell death by pyroptosis (as does activation of caspase-1)
- Pyroptosis is mediated by caspase-1 or -11 induced cleavage of Gasdermin D
  - Shi et al Nature (2015) doi:10.1038/nature15514,
  - Kayagaki et al Nature (2015) doi:10.1038/nature15541)

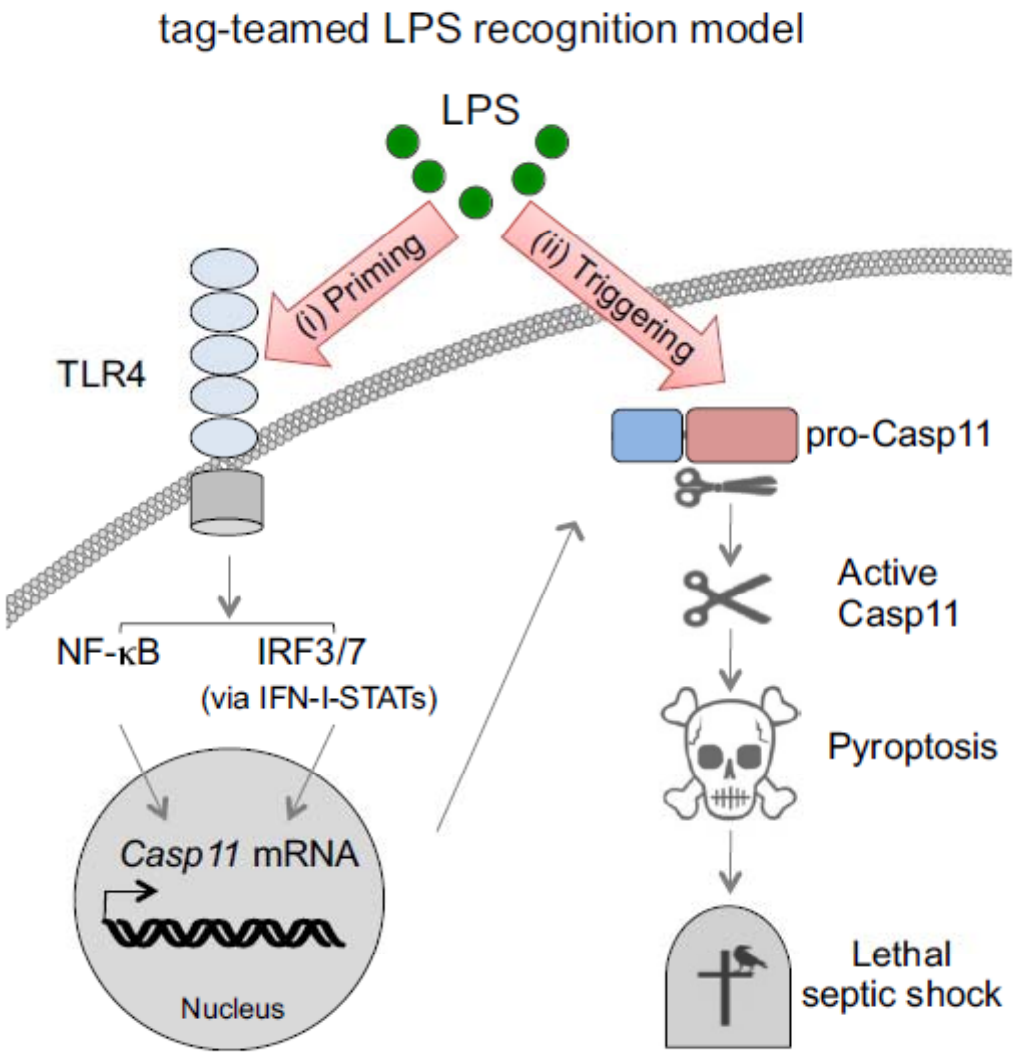
Cytoplasmic sensing of LPS – Gasdermin D



Gasdermin D is cleaved at a conserved DG motif (D276 in mouse) in a recognition sequence that differs from that classically recognised by caspases

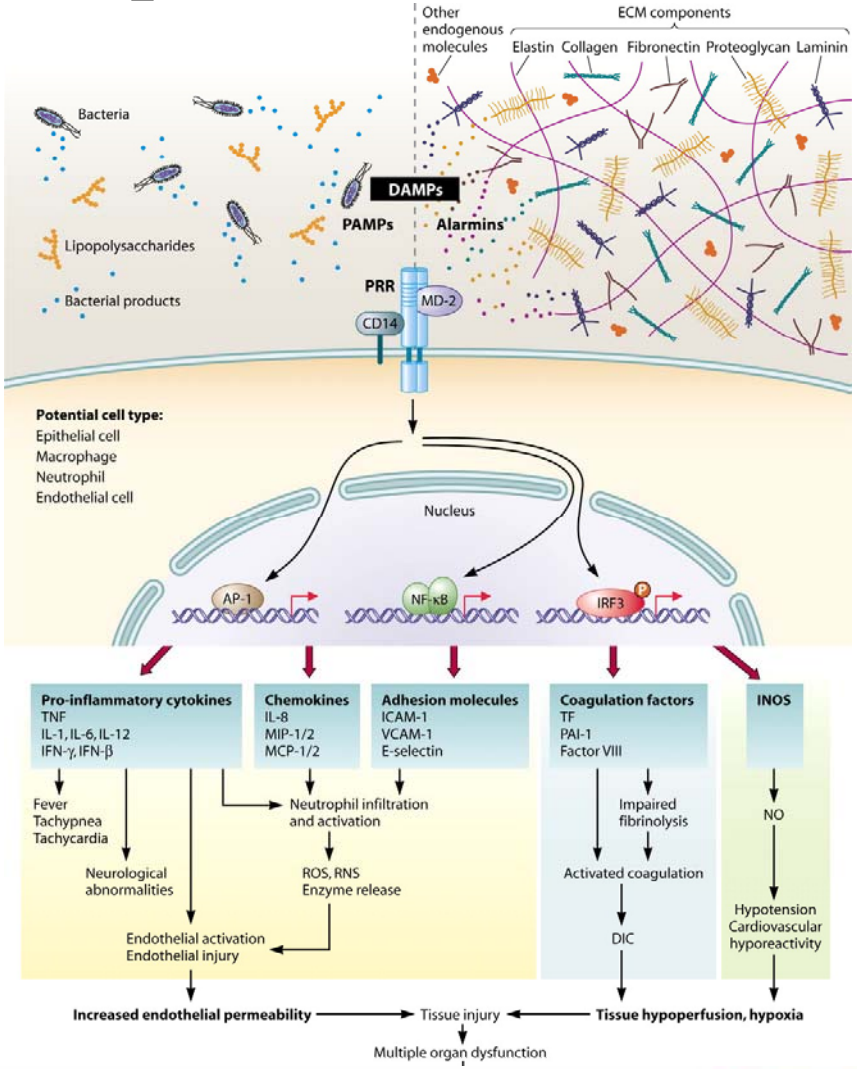
Broz Nature (2015) doi:10.1038/nature15632

Cytoplasmic sensing of LPS – Caspase-11



Stowe et al 2015 Immunol Rev 265:75-84

The major pathway and inflammatory mediators of sepsis and its related conditions.



Ineke Vanlaere, and Claude Libert Clin. Microbiol. Rev. 2009;22:224-239

Clinical Microbiology Reviews

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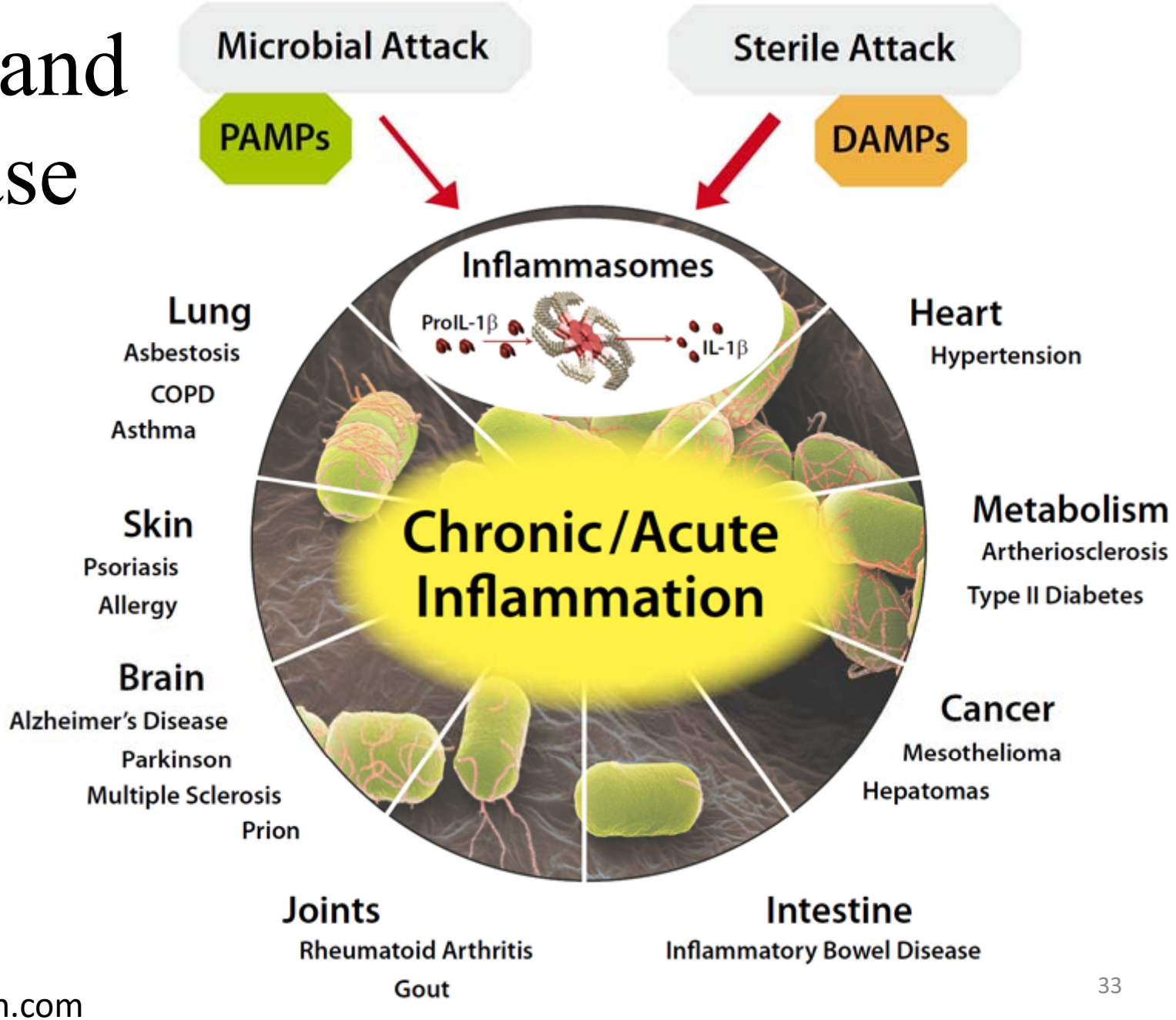
PRRs and disease



www.adipogen.com

TLR SNPs and infection

NLRs alone connected to >50 diseases

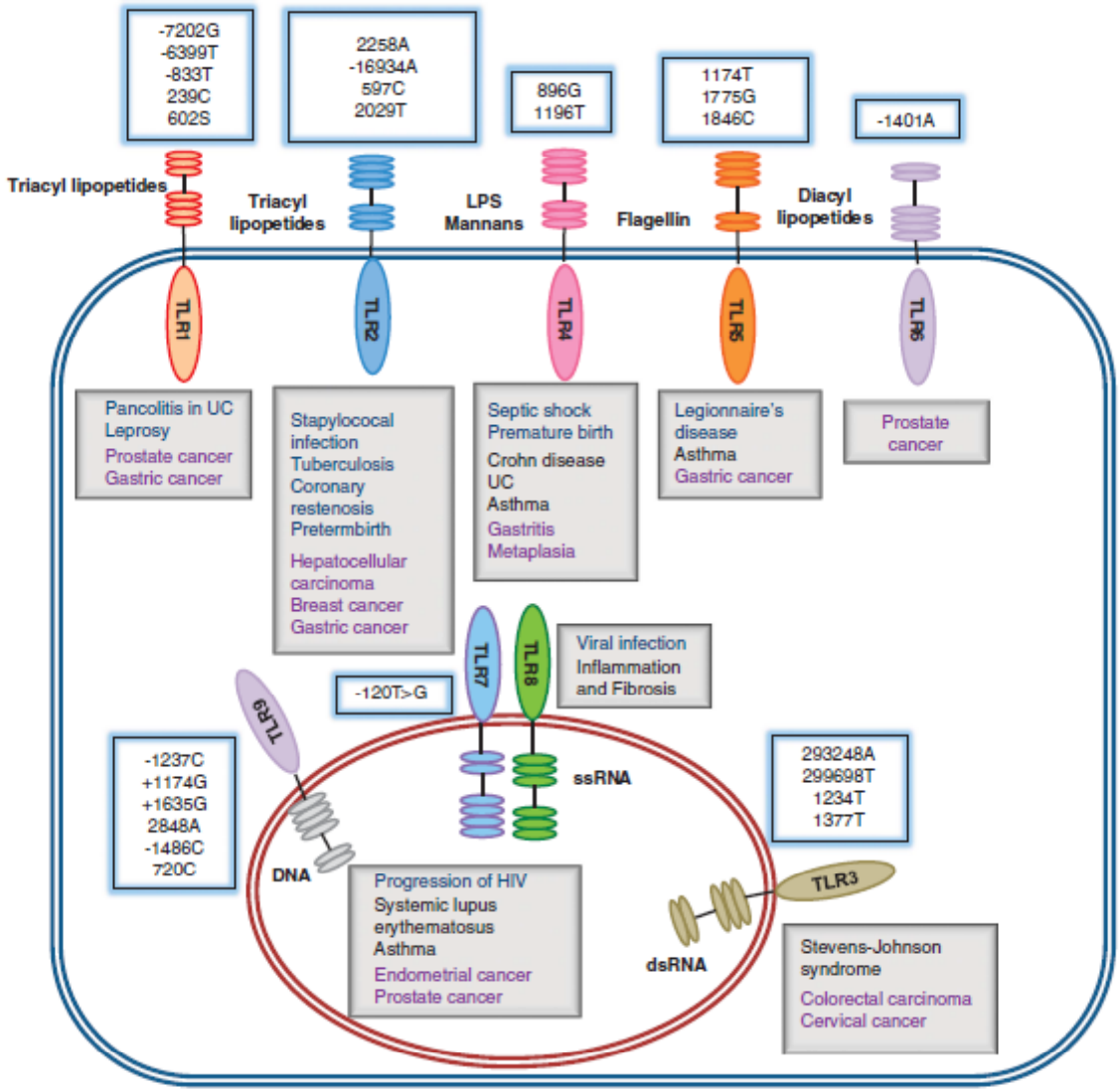


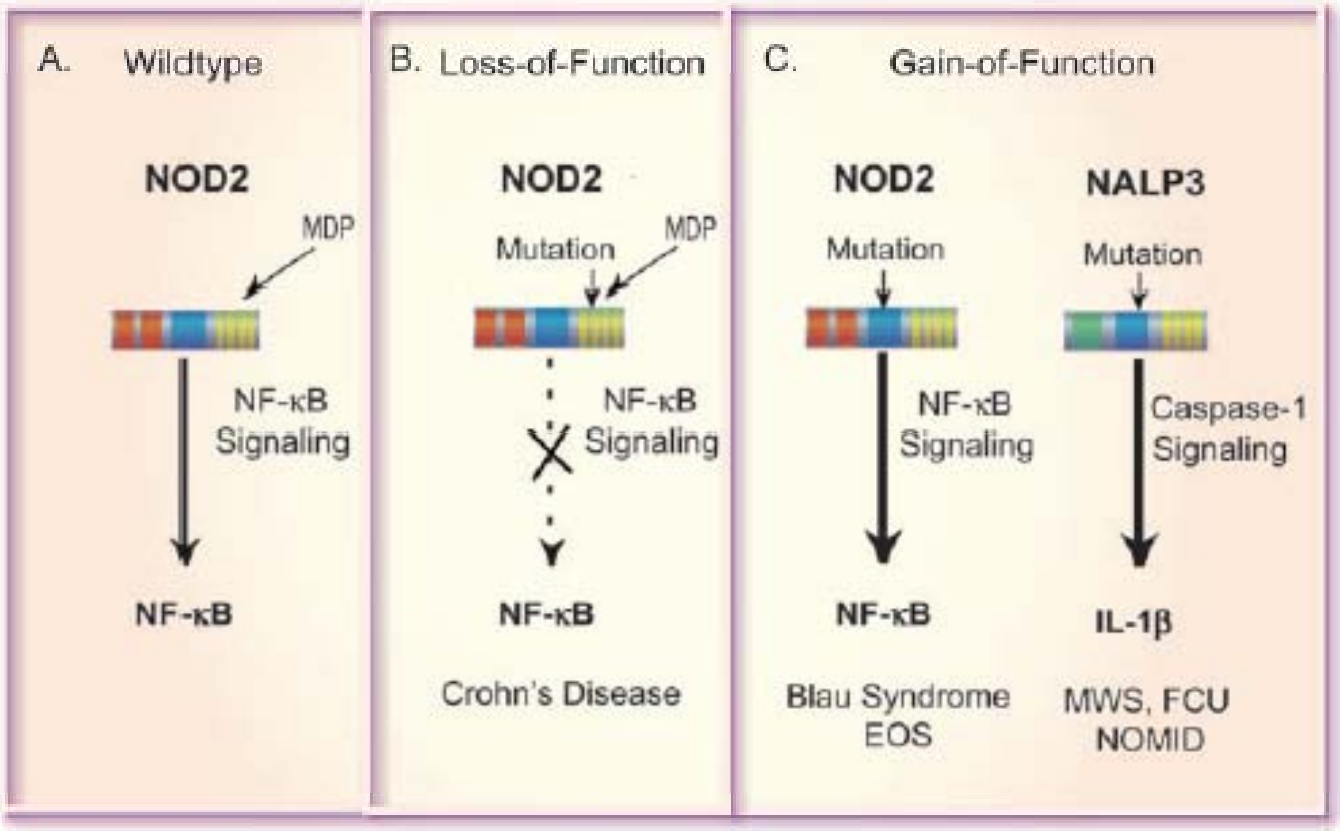
Figure 2. Genetic association between TLRs and susceptibility to infections, inflammatory disease and cancer. dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; UC, ulcerative colitis.

Trejo et al *Genes and Immunity* (2014) **15,** 199–209

NLR polymorphisms and disease

NOD2 CD:
R702W
G908R
L1007fsincC

NOD2 Blau:
R334Q



NLRP3 MWS:
R260W
A352W
G569R

NLRP3 FCAS:
V198M
A439V
E627G

Gain of function polymorphisms often lead to receptor autoactivation by disrupting the inactive conformation

Wilmanski 2008 Journal of Leukocyte Biology vol. 83 no. 1 13-30